DEVELOPMENT OF TARGETED DRUG DELIVERY SYSTEM FOR BREAST CANCER

SYNOPSIS

BY

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Introduction

Cancer is amongst the leading causes of death worldwide and approximately 70% of deaths occur in low and middle-income nations. Breast cancer is the most frequent cancer in women, causing morbidity or mortality in the developing as well as in developed nation. Nowadays, the main practice for breast cancer treatment is surgery followed by chemotherapy or/and radiation therapy (1). Most frequently prescribed chemotherapeutic agent for the treatment and management of breast carcinomas is Paclitaxel (PAX). PAX acts through unique mechanism of enhancing polymerization of tubulin, which further hyper-stabilizes the microtubules, leading to obstruction in cell propagation and induces programmed cell death(2-4). PAX is a hydrophobic drug with poor solubility in water (0.3–0.5 µg/mL), for this reason, it is commonly formulated as Taxol®, a market formulation composed of a mixture of Cremophor EL® and dehydrated alcohol (1:1, v/v). Cremophor is associated with a variety of side effects such as hypersensitivity, nephrotoxicity, neurotoxicity and hypotension (4, 5) .

Nanoparticulate systems have been studied for several decades offering an advantageous alternative to the use of toxic excipients to improve drug solubility and favour a controlled drug release. Drug-loaded nanoparticles (NPs) can escape from the vasculature through the leaky endothelium overlying the tumour and then accumulate preferentially in many types of solid tumours by the phenomenon known as Enhanced Permeability and Retention (EPR) effect (6). Various PAX formulations such as liposomes (7), solid lipid nanoparticles (8), polymeric NPs (9), polymeric micelles and drug conjugates (10, 11) have been examined to achieved certain improvements in PAX solubility.

To overcome the Cremophor associated side effect and improve the solubility of PAX, the Food and Drug Administration (FDA) in 2005 approved a albumin-bound PAX-NPs formulation (Abraxane®) to treat metastatic breast cancer (12). However, it suffers from a major flaw that is rapid elimination of PAX and hence reduced bioavailability (13). Further, being an expensive formulation limits its use in low- and middle-income countries. Even though, PAX is effective alone, it is frequently used in combination with other drugs (mainly anthracycline-based chemotherapy regimens) to reduce its side effects and enhance its cytotoxicity against breast cancer. These combinations come at a cost of increased nonspecific cytotoxicity such as, doxorubicin induced cardiotoxicity; docetaxel induced bone marrow suppression and peripheral neurotoxicity; cyclophosphamide induced neutropenia etc (14). Likewise, other chemotherapeutic agents (cisplatin, 5-fluorouracil, mitoxantrone, etc.) are also known to induce non-specific toxicity. The answer to this prevailing problem is conjoining PAX with some other potent and nontoxic moieties.

In the present study, we aimed to reduce the toxicity of the PAX by adopting the two different approach, firstly combinational approach and secondly by developing targeted drug delivery system. Clotrimazole (CLZ) is a clinically used antifungal drug, possessing profound anti-proliferative effect on tumorigenic and metastatic cells while having minimal effect on normal cells (15). CLZ acts by inhibiting major glycolytic regulatory enzymes viz. 6-phosphofructo-1-kinase (PKF) and hexokinase (HK), which are involved in cancer biology, by altering glucose metabolism and energy production. More importantly, CLZ is known to induce cell cycle arrest at G1- and M-phase (16). Increased glucose utilization and aberrant glucose metabolism during proliferation phase is a hallmark of cancer. Increased fermentative glycolytic flux is the distinguishing characteristic possessed by cancer cell, even with the high oxygen supply, a phenomenon known as "Warburg effect". As a consequence of the Warburg effect, there is over activation of PFK, which confers several advantages for the growth and invasion of tumour cells (17). Studies suggest that by blocking glycolysis there is inhibition in tumour progression and furthermore enhances the efficacy of chemotherapy (18). By arresting the cell cycle and inhibiting glucose metabolism, co-joining CLZ with PAX might provide useful biochemical rationale for the treatment of breast carcinoma.

Targeted drug delivery system (TDDS) or smart drug delivery system is a unique method of delivering medication to a patient in such a manner that it increases the concentration of drug only in the desired part of the body (like cancerous organ) and it is also called as active targeting. Surface modification of NPs by conjugating targeting moiety is one such TDDS that allows longer blood-circulation and favours NPs preferential accumulation in solid tumours by active targeting and it also enable us with the advantageous benefits of EPR effect (19).

Our second approach was to mount active targeting peptide on the surface of polymeric NPs, for which we have selected Sialic acid (SA)/poly-sialic-acid (PSA). It is an endogenous electro-negatively charged monosaccharide moiety present in higher animal. PSA is biocompatible, non-toxic, non-immunogenic and biodegradable. Apart from these, it can serve as targeting moiety for selectins (E-selectin/P-selectin) which are overexpressed in the tumour vascular endothelial cells. PSA conjugated NPs can transport drug directly into the vicinity of tumour through receptor–mediated endocytosis and increase the potency of drug (20, 21). Meanwhile, PSA degradation yields CO_2 and water that are non-toxic to living organisms. Literature suggests numerous techniques that have been attempted to conjugate sialic acid to the surface of polymeric NPs. Carbodiimide crosslinking methodology is a successful and efficient technique to conjugate protein moiety on the surface of polymeric NPs (22). It possesses a special feature of zero-length cross-linking by establishing stable amide bond between

carboxylic acid group and amine group and may benefit further by retaining the dimensions of NPs in nano scale (23).

Taking all these facts into consideration present study was designed with following objectives:

Objective-1: To evaluate the effect of paclitaxel and clotrimazole combination against breast cancer cell line.

Objective-2: To formulate and characterize sialic acid conjugated nanoparticles.

Objective-3: To optimize drug loaded polymeric nanoparticles.

Objective-4: To synthesis sialic acid drug loaded nanoparticles and evaluation against breast cancer cell line.

Objective 1

OPTIMIZATION OF PACLITAXEL (PAX) AND CLOTRIMAZOLE (CLZ) CONCENTRATION.

Compatibility analysis through fourier transform infrared spectroscopy

The FTIR spectra of PAX depicted its prominent and characteristic peaks at 3312/cm (O-H and N-H stretching), 2974/cm (C-H stretching), 1704/cm (COOH stretching) and 1248/cm (C-N stretching). For CLZ, characteristic IR peaks at 1495/cm and 1216/cm (C-N stretch) and 769/cm (C-Cl stretch) were observed. Representative band peaks of two drugs appeared with no substantial interaction in the physical mixture of PAX-CLZ revealing stability of two compounds without any chemical interface.

Cell proliferation assay and selection of drug concentration

The effect of drug treatment on MCF-7 cells (breast cancer cell line) was evaluated with various doses of PAX and CLZ individually. Both PAX and CLZ exhibited dose dependent cytotoxicity with IC_{50} values of 53.68 nM and 51.52 µM respectively. Further, we explored the combination (combo) of PAX and CLZ in varying concentrations and these concentrations were designed in such a way that they did not outpace individual IC_{50} values of PAX and CLZ. We observed 50% decline in cell viability with combo of 12.5 nM PAX and 25 µM CLZ, nevertheless, a reduced cell viability with approximate 25% reduction was recognised for PAX (12.5nM) and 30% for CLZ (25µM) individually. This finding revealed that, combination is profoundly cytotoxic to cancer cells in contrast to individual drug treatment.

To determine additive, synergistic or antagonistic response, we estimated combination index (CI) through CompuSyn Software. Interactions between PAX and CLZ against MCF-7 cells were graphically represented in dose effect curve. Dose reduction index (DRI) advises what percent fold reduction of the dose for each drug within the combination, can be reduced to generate a specific effect as a result of synergism. DRI>1 indicate favourable dose reduction, were as DRI <1 and =1 represent unfavourable dose-reduction and no dose-reduction correspondingly. It should be noted that DRI is associated with CI, however solely CI values will efficiently verify synergism or antagonism (24). It should to be noted that once the dose of a drug is reduced, toxicity of the drug will eventually decrease (24). Taking this fact into consideration and also the results revealed through FA-CI plot (Chou-Talalay Plot), we nominated PACL as PAX 12.5 nM and CLZ 25 µM combination (0.66 CI value- representing synergism) for further experimental analysis.

Anti-cancerous effect of PACL was also observed on MDA-MB-231 cells. Additionally, PACL showed no significant cytotoxic symptoms on normal epithelial HEK-293 cells. These

observations suggest that combo is acting synergistically at preferred concentrations against cancer cells while exhibiting minimal effect on normal cells.

Acridine orange (AO)/ethidium bromide (EtBr) live dead staining

The nuclear morphology of MCF-7 and MDA-MB- 231 cells were examined by AO and EtBr. The cells were treated with PAX, CLZ and PACL for 24 h and compared with the untreated cells. Cells stained with green fluorescence represent viable and healthy state, however yellow stain is a characteristic feature of early apoptosis and orange or reddish stain signifies late apoptosis. In the control group, uniformly green live cells with normal and large nucleus morphology were observed, whereas in cells treated with combo, yellow, orange and red staining was observed. Further, mean fluorescence intensity was determined for red colour (EtBr stained dead cells) by using Image-J software. Meanwhile treatment with PACL significantly increased fluorescent intensity in MCF-7 (22.4-fold) and MDA-MB-231 (13.8-fold) when compared to control cells. However, individual performance of both the drugs was minimal when compared to combo. PAX (12.5 nM) caused non-significant increase in fluorescence intensity for MCF-7 (1.8-fold) and MDA-MB-231 (1.51-fold) compared to control. Similarly, CLZ (25μM) treatment showed slight increase in fluorescence intensity (9.06-fold for MCF-7 cells and 4.9-fold for MDA-MB-231 cells). These results suggest that the PACL has significantly high anti-cancer activity as compared to individual drug treatments.

Comet assay

We performed comet assay to analyse the DNA damage, a consequence of individual drug treatment or in combination. Fragmented DNA molecule migrates under the influence of constantly applied electric field and smaller the DNA molecule, faster is its movement. Undamaged DNA appears as a head and migrating DNA fragments represent the characteristic appearance of comet tail. Greater the length of tail, greater is the DNA damage and vice-versa. We observed a significant growth in DNA damage, by PACL treated MCF-7 and MDA-MB-231 cells with 50% and 73% respectively. Whereas, individual drug treatment exhibited significantly less DNA damage with PAX showing 18% and 44% while CLZ exhibiting 34% and 61% for MCF-7 and MDA-MB-231 cells respectively. These results verified that the PACL is synergistically effective against cancerous cells in comparison to single drug treatments.

Glucose consumption

There is always an overburden of glucose in case of cancer cells in comparison to normal tissue and any halt in glucose uptake causes cellular insult. Moreover, CLZ is known to inhibit glucose uptake preferentially in cancer cells rather than normal cells. Hence, we investigated

the glucose consumption upon incubating the cells (MCF-7 and MDA-MB-231) with PAX (12.5nM), CLZ (25µM) and PACL (PAX-12.5nM, CLZ -25µM). Interestingly, PACL exhibited glucose uptake only of 15% in MCF-7 and 20% in MDA-MB-231 cells (Fig.6). Whereas the cells treated with PAX (12.5nM) showed glucose uptake near about 70% in MCF-7 and 60% in MDA-MB-231 cells. Likewise, CLZ showed 45% glucose uptake in MCF-7 and 50% uptake in MDA-MB-231. It is clear from the presented data, that PACL is more effective in inhibiting the cell glucose uptake in comparison to individual drug treatment.

Conclusion

It is evident by the obtained results from cytotoxicity, CompuSyn software analysis and live dead staining that the combination of PAX 12.5nM and CLZ 25 µM (PACL) acts synergistically toward breast cancer cells by inducing genotoxicity and reducing glucose uptake.

Objective 2

FORMULATION AND CHARACTERIZATION OF SIALIC ACID (SA) CONJUGATED NANOPARTICLES.

Fabrication of polymeric nanoparticles and sialic acid conjugated nanoparticles

Paclitaxel encapsulated polymeric nanoparticles (PCL-NPs) were synthesized by employing o/w emulsion and solvent evaporation technique. Likewise, blank and rhodamine (Rhd-0.01mg/ml) loaded PCL-NPs were also fabricated. Sialic acid conjugated nanoparticles (SA-PCL-NPs) were formulated via mounting SA on PCL-NPs through carbodiimide reaction (EDC + NHS).

Table 1: Formulation Table

Formulation Code	PCL (mg)	PVA	Drug(mg)	Conjugation	Entrapment Efficiency (%)
PCL-NPs	100	1%	-	-	-
SA-PCL-NPs	100	1%	-	SA	-
PCL+PAX-NPs	100	1%	PAX (5)	-	34.4 ± 2.3
SA-PCL+PAX-NPs	100	1%	PAX (5)	SA	

Characterization

Scanning electron microscopy, particle size distribution, zeta potential

Surface morphology of the NPs was examined through scanning electron microscopy (SEM) and observation revealed that the particles were isolated, discrete and spherical in shape with uniform size distribution. The particles size and zeta potential analysis was performed using zetasizer (Nano ZS 90, Malvern Instruments Ltd., Malvern, UK). Where, we observed slight increase in the size of SA-PCL-NPs (175.6 ± 1.98 nm) in contrast to PCL-NPs (169.5 ± 4.67 nm) and also, we witnessed increase in the surface charge with SA-PCL-NPs in comparison to PCL-NPs. This may be attributive to SA mounting on the surface of PCL-NPs.

Fourier-transform infrared spectroscopy, Ultraviolet-Visible Spectroscopic and X-Ray Diffraction analysis

FTIR-spectral interpretation was performed to evaluate the surface modification of PCL-NPs. Characteristic peaks for sialic acid were; O-H stretch at 3282cm^{-1},1644cm^{-1} representing C=O and 1518cm^{-1} depicts the N-H bending. PCL-NPs represents the specific C-H stretching at 2948cm^{-1} and 1726cm^{-1} represents carboxylic acid C-O strong peak. FTIR spectra of SA-PCL-NPs exhibited the strong amide peaks at 3282, 1644 and 1518 cm^{-1} representing the O-H, C=O and N-H peaks respectively, suggesting SA conjugation on the surface of PCL-NPs. To further investigate the conjugation ultraviolet spectrophotometric analysis was performed. SA-PCL-NPs represents the strong peak at 280 nm which corresponds to the protein absorption peak arising due to the aromatic ring presence. Whereas, protein peak was found to be absent in the PCL-NPs. XRD pattern of the PCL-NPs showed the major strong peaks specific for PCL at 2θ =21.5° and 23.8°. After being conjugated with sialic acid, peaks became slightly broader and weaker in contrast to PCL-NPs, implying low crystallinity as an evidence for the presence of SA on the surface. FTIR analysis, UV-spectral investigation and XRD analysis assured the sialic acid mounting on the surface of the PCL-NPs.

Drug release studies

Drug release studies were performed to investigate whether SA mounting on the surface of PC-NPs is having any consequences with respect to drug release or not. Results revealed no alteration in drug release pattern with either of the formulation (PCL-NPs and SA-PCL-NPs).

Cytotoxicity studies

MTT assay was performed to evaluate the percentage cell inhibition as an effect of drug loaded (PCL-PAX-NPs and SA-PCL-PAX-NPs) nanoparticles. Cells (HEK 293, MCF 7 and MDA MB 231) were incubated with fabricated nanoparticles (PCL-NPs, SA-PCL-NPs, PCL-PAX-NPs and SA-PCL-PAX-NPs) for different time periods (0 to 48h). SA-PCL-PAX-NPs were found to be most effective against cancer cell lines and exibeted time dependent cytotoxicity.

PCL-NPs and SA-PCL-NPs did not exhibit any sign of cell cytotoxicity and hence provided us an indication that sialic acid conjugation have no effect on normal cells. Highest cell killing capability was observed with sialic acid functionalized drug loaded NPs and this is possibly because of sialic acid driven cellular uptake. Less cell toxicity was observed with PCL-PAX-NPs in contrast to SA-PCL-PAX-NPs.

Microscopic analysis of cellular uptake

To validate the cell specific uptake, Rhodamine (Rhd) encapsulated NPs were further formulated using similar fabrication and conjugation methodology. Fabricated Rhd-NPs and SA-Rhd-NPs were exposed to normal (HEK-293) and cancerous (MCF-7 & MDA-MB-231) cells. DAPI-fluorescent dye was employed to stain nucleus of the cells and images were capture through inverted fluorescent microscope. Captured images were further subjected to Image-J software analysis and mean fluorescent intensity for red fluorescence was calculated. Data was represented as corrected total cell fluorescence (CTCF) in graphical form. Where, SA-Rhd-NPs were successfully homed inside the cancer cells in comparison normal cells. These observations were further confirmed through CTCF values and the graphs revealed that cancer cells were having higher red fluorescent intensity (rhodamine red fluorescence) than that of normal cells, suggesting SA had facilitated the NPs uptake in cancer cells.

CONCLUSION

Characterization reveals spherical & smooth surface morphology of synthesized nanoparticles. Sialic acid was successfully mounted on the surface of polycaprolactone nanoparticles as revealed through particle size distribution and zeta potential of nanoparticles. Fourier-transform infrared spectroscopy, utraviolet-visible spectroscopy and X-Ray Diffraction further confirmed the sialic acid conjugation on the surface of nanoparticles. Sialic acid conjugated nanoparticles exhibited tumor specificity and were found to be less-toxic to normal cells.

Objective 3

OPTIMIZATION OF DRUG LOADED POLYMERIC NANOPARTICLES

Preliminary investigation

Preliminary investigation was performed, to identify the independent variables that might affect the eminence of nano-formulation and was aimed to enhance the desired quality attributes of nanoparticles such as particle size (PS) and entrapment efficiency (%EE). Polymer: drug ratios, concentration of surfactant (PVA) and organic phase: aqueous phase ratios were found to most critical independent variables responsible for the stability of nano-

formulation. Further the working range for each variable were selected by considering the facts, that the particle size should not exceed 200nm and % EE should be greater than 40 %. The effects of selected variables on the PS and % EE were studied to calculate their optimal values for optimization of formulation using response surface experimental design and finally optimized through Box Behnken Design.

Response surface methodology and Box-Behnken design

Box-Behnken design (BBD) is a response surface methodology (RSM) involving a group of statistical and mathematical methods useful for the modelling and analyzing problems associated with method or experimental work. It serves as a tool to optimize the response that is influenced by various parameters/factors either related to process or formulation. It also quantifies the relationship between the independent parameters/factors and the obtained response surfaces. In the present investigation, a three variable BBD-RSM was used to study the effect of polymer: drug ratio, organic phase: aqueous phase ratio and surfactant concentration on PS and %EE of PCL-PAX-NPs. BBD generated total 15 set of experimental runs and all the resulting formulations were further investigated for PS distribution followed by % EE.

Effect of variables on particle size(PS) studied through (BBD)

The PS varied from 176.4 nm (F3) to 266.8 nm (F2) which shows that combination of critical formulation variables affects the PS of NPs. From the statistical analysis, we observed that the R^2 value for actual v/s predicted values of PS was 0.92 which was a good correlation. The results of ANOVA demonstrated that the selected model was significant for effect of selected variables on PS with p value < 0.05. The obtained results were further analyzed through 3D surface graphical plot, where we observed that with the increase in polymer: drug ratio there was increase in PS of NPs. In case of polymer: drug ratio and aqueous: organic ratio, no prominent effect was observed from 3D plot. When surface plot for surfactant concentration and aqueous: organic phase was looked at, decline in PS was witnessed.

Effect of variables on entrapment efficiency (%EE) studied through (BBD)

The %EE for each formulation batch was recorded, that varied from 34.4% (F14) to 64.6% (F2) which further suggested that the various combinations of critical formulation variables affected the %EE of NPs. From statistical analysis R^2 value for actual v/s predicted values for %EE was found to be 0.94 which was a good correlation. The result of ANOVA confirmed that the model was significant for effect of selected variables on %EE with p value < 0.05. Polymer: drug ratio and surfactant concentration 3D surface plot reveled that, increase in

polymer: drug ratio will increase EE%. Likewise, rise in aqueous: organic phase will increase EE% as exhibited by polymer: drug and aqueous: organic surface plot analysis. Whereas, increment in surfactant concentration will decrease % EE as reveled by aqueous: organic and surfactant concentration 3D surface plot.

Optimization of variable through desirability plot

Desirability plot was generated using Design Expert software, where the upper and lower desired values of response were selected and software generated the values of variables which would help to achieve the desired response (PS < 200nm and %EE ≥ 40) . A composite desirability of 0.924 was obtained which predicts that if the formulation is prepared according to the optimized values of independent variables, the chance to obtain desired value of response is 92.4%. The suitability of desirability plot was assessed by synthesizing a formulation of suggested batch and comparing it with predicted response. The obtained value of response was very close to predicted values for responses. This shows that the optimized formulation predicted by software holds true for practical purpose.

CONCLUSION

Three different independent variables; polymer: drug ratios, concentration of surfactant (PVA) and organic phase: aqueous phase ratios were optimized by using response surface methodology (RSM) – Box Behnken design (BBD). The obtained results demonstrated the establishment of a suitable and robust design to formulate nano-formulation and these optimized variable concentrations can be employed further for scale up formulation studies.

Objective 4

SYNTHESIS OF SIALIC ACID DRUG LOADED NANOPARTICLES AND EVALUATION AGAINST BREAST CANCER CELL LINE.

Fabrication of drug loaded PCL-nanoparticles

To match the optimized organic: aqueous ratio (1:3) and surfactant concentration (1%), 2ml of dichloromethane (DCM) was used as organic phase and 6ml of 1% polyvinyl alcohol (PVA) was used as aqueous phase. 500 mg of polycaprolactone (PCL) as polymer and 50 mg of drug was preferred to match optimized polymer to drug ratio. 25µg of PAX and 50mg of CLZ was further selected to comply with optimized drug combination (12.5 nM of PAX and 25µM of CLZ). Dug loaded PCL-NPs were synthesized by o/w emulsion and solvent evaporation technique. Sialic acid conjugated nanoparticles (SA-PCL-NPs) were formulated via mounting SA on PCL-NPs through carbodiimide reaction (EDC + NHS).

Table 2. Optimized formulation table

Formulation	PCL (mg)	PVA	Drug	PS (nm)		EE (%)	
				Unconjugated	SA-conjugated		
PCL-NPs	500	1%	-	184.4 ± 2.8	187.4 ± 1.3	-	
PAX-NPs	500	1%	D1	189.6 ± 3.4	191.6 ± 1.9	59.45 ± 1.8	
CLZ-NPs	500	1%	D2	188.2 ± 2.7	193.2 ± 2.6	63.37 ± 2.6	
PACL-NPs	500	1%	D1+D2	192.5 ± 2.8	197.5 ± 2.8	D1	D2
						50.91±1.8	47.54±3.1

D1 = Paclitaxel (12.5nM) **PCL** = Polycaprolactone **PS** = Particle Size

D2 = Clotrimazole (25μM) **PVA** = Polyvinyl Alcohol **EE** = Entrapment Efficiency

Characterization

Scanning electron microscopy, particle size distribution and % entrapment efficiency

Scanning electron microscopic (SEM) analysis revealed the spherical and smooth surface morphology of formulated NPs. The particles size distribution analysis suggested that all formulations were having PS less than 200nm (Table 2). EE % of the drug loaded NPs was calculated according to the equation % EE= (weight of drug in nanoparticles/weight of the feeding ratio) × 100. Where, SA-PACL-PCL-NPs exhibited 50.91 ± 1.8 of %EE for PAX and 47.54 ± 3.1for CLZ respectively (Table 2). Likewise, 59.45 ±1.8 %EE was calculated for SA-PAX-PCL-NPs and 63.37 ± 2.6 for SA-CLZ-PCL-NPs simultaneously.

Fourier-transform infrared spectroscopy

FTIR-spectral interpretation was performed to check the surface modification of PCL-NPs. Characteristic peaks for SA; O-H stretch at 3282cm^{-1},1644cm^{-1} representing C=O and 1518cm^{-1} depicts the N-H bending. PCL-NPs represents the specific C-H stretching at 2948cm^{-1} and 1726cm^{-1} represents carboxylic acid C-O strong peak. FTIR spectra of SA-PCL-NPs exhibited the strong amide peaks at 3282, 1644 and 1518 cm^{-1} representing the O-H, C=O and N-H peaks respectively, suggesting SA conjugation on the surface of PCL-NPs.

Drug release studies

Drug release studies were performed at two different pH; 6.8 pH (mimics the pH of breast cancer) and 7.4 pH (normal pH level of blood). Where we witnessed slight increase in the drug release at lower pH, but important point to note, that over all drug release pattern was found to be in controlled proportion for both the compounds (PAX and CLZ).

In-vitro cell cytotoxicity studies

The cellular cytotoxicity of synthesized NPs was studied through Mtt assay on MCF-7 and MDA-MB-231 breast cancer cell lines and HEK-293 normal cell lines. Cells were incubated with formulated NPs (PCL-NPs, SA-PAX-NPs and SA-CLZ-NPs and SA-PACL-NPs) for different time periods (0 to 72h). Study demonstrated that SA-PCL-PACL-NPs were most effective against cancer cell lines in comparison to PCL-PACL-NPs, where the cellular growth inhibition was greatly influenced by incubation time period. PCL-NPs and SA-PCL-NPs did not exhibit any sign of cell toxicity. Highest cytotoxicity was observed with SA-PCL-PACL-NPs and the apparent cause of such behaviour is because of SA driven cellular uptake.

Acridine orange (AO)/ethidium bromide (EtBr) live dead staining

To authenticate the cell cytotoxicity, nuclear morphological changes were examined by performing live dead staining experiment on HEK-293, MCF-7 and MDA-MB- 231 cell lines. Cells stained with green fluorescence represent viable and healthy state, however yellow stain is a characteristic feature of early apoptosis and orange or reddish stain signifies late apoptosis. Further, mean fluorescence intensity was determined for red colour (EtBr stained dead cells) by using Image-J software. We witnessed no significant sign of cell apoptosis in normal cells (HEK-293) where as to cancerous cells (MCF-7 and MDA-MB-231) exhibited prominent cell apoptosis which was further confirmed through Image j analysis.

CONCLUSION

Optimized nano-formulation was found to be cancer specific side by side having its minimal effect on normal cells.

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2. **Arun Sharma,** Vineet Mehta, Arun Parashar, Udayabanu Malairaman. "Combinational effect of Paclitaxel and Clotrimazole on human breast cancer: Proof for synergistic interaction". Synergy;2017 (5) 13-20 [Scopus Indexed].
3. **Arun Sharma,** Vineet Mehta, Arun Parashar, Riddhi Patrwal, Udayabanu Malairaman. "Solid Lipid Nanoparticle: Fabricated through nanoprecipitation and their physicochemical characterization". International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 8(10)1- 5 [Scopus Indexed].
4. **Arun Sharma,** Aniket Sood, Vineet Mehta, Udayabanu Malairaman. "Formulation and Physicochemical evaluation of nanostructured lipid carrier for codelivery of clotrimazole and ciprofloxacin". Asian Journal of Pharmaceutical and Clinical Research. 2016; 9(3) 1-5. [Scopus Index].
5. **Arun Sharma,** Vineet Mehta, Gopal Singh Bisht, Udayabanu Malairaman. "Polymeric nanoparticles-mediated delivery system for anti-bacterial Peptides". International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 8(3)45. (Abstract Published) [Scopus Indexed].

ASSOCIATED PUBLICATION

1. Kumari A, **Sharma A**, Malairaman U, Singh RR. (2018) Proficient surface modification of CdSe quantum dots for highly luminescent and biocompatible probes for bioimaging: A comparative experimental investigation. Journal of Luminescence. 1; 199:174-82 Scopus Indexed (IF-2.6).

2. Patel SS, Ray RS, **Sharma A**, Mehta V, Katyal A, Udayabanu M (2018). Antidepressant and anxiolytic like effects of Urtica dioica leaves in streptozotocin induced diabetic mice. Metabolic brain disease. 27:1-2 Scopus Indexed (IF-2.2).

3. Mehta V, Parashar A, **Sharma A**, Singh TR, Malairaman U. (2017) "Quercetin ameliorates chronic unpredicted stress-mediated memory dysfunction in male Swiss albino mice by attenuating insulin resistance and elevating hippocampal GLUT4 levels independent of insulin receptor expression". Hormones and Behavior.;89:13-22. [Frank A. Beach Award Paper] Scopus Indexed (IF-3.3)

4. Sharma S, **Sharma A**, Mehta V, Chauhan RS, Malairaman U, Sood H. (2016) efficient hydroalcoholic extraction for highest diosgenin content from trillium govanianum (nag chhatri) and it's in vitro anticancerous activity. AJPCR, 4(9): 1-7 . Scopus Indexed (IF-0.49)

5. Mehta V, **Sharma A**, Tanwar S, Udayabanu M. (2016). In-vitro and in-silico evaluation of the antidiabetic effect of hydroalcoholic leaf extract of Centella asiatica. International Journal of Pharmacy and Pharmaceutical Sciences, 8, 257-362. Scopus Indexed (IF-0.51)

6. Bhardwaj V, Monga J, Sharma S, **Sharma A**, Sharma A and Sharma P. (2014). Novel surfactant immobilized micellar system of oxidation inhibitors in clotrimazole bio-adhesive gel formulation: Development, characterization, in vitro and in vivo antifungal evaluation on Candida clinical isolates. RSC Adv., 4, 47207-21. Scopus Indexed (IF-3.1)

7. Sharma L and **Sharma A**. (2014). Invitro antioxidant, anti-inflammatory and antibacterial activity of Withania somnifera. Journal of Chemical and Pharmaceutical Sciences, 6(7):178-82. Scopus Indexed.

CONFERENCE AND WORKSHOPS

1. **Arun Sharma** and Udayabhanu Malairaman. Concept of drug repositioning and its application in breast cancer. [International Conference on "Challenges in clinical trials and pharmacovigilance of new drugs, genetically modified products and herbal drugs" on 28th & 29th March 2018]. **Received 2nd Prize in oral Presentation**
2. **Arun Sharma,** Riddhi Patrwal, Gopal Bisht, Udayabhanu Malairaman. (2015) Nanoparticles-mediated delivery of the antimicrobial peptide against bacterial infection. [International Conference 4th Nanotoday Conference, JW Marriot Marquis Dubai, **United Arab Emirates**: 6-10 December 2015].
3. **Arun Sharma**, Vineet Mehta, Arun Parashar, Udayabanu Malairaman (2016). Polymeric nanoparticles-mediated delivery system for anti-bacterial peptides. International Conference on Innovations in Pharmaceutical Sciences. [Sri Aurobindo Institute of Pharmacy, Indore, Madhya Pradesh, India: 27-28 February 2016].
4. **Arun Sharma**, Vineet Mehta, Udayabanu Malairaman (2014). In-vitro and IN-vivo evaluation of solid dispersion of Rosuvastatin. International Conference on Pharmaceutical Sciences- Present Trends and Future Prospects in Pharmaceutical Sciences [Shri Guru Ram Rai Institute of Technology & Science, Dehradun, Uttarakhand, India: 14 - 15 February 2014].
5. **Arun Sharma** (2016). National Symposium on Computational Systems Biology (NSCSB). [Jaypee University of Information Technology, Waknaghat, Himachal Pradesh, India: 18-20 March 2016]
6. **Arun Sharma** (2016). Workshop on Statistical Techniques in Biological and Medical Sciences (STBMS). [Jaypee University of Information Technology, Waknaghat, Himachal Pradesh, India: 13-18 June 2016].
7. **Arun Sharma**, Varun Bhardwaj, Udayabanu Malairaman (2014) National Conference on "Recent Advancement in Novel Drug Delivery System and Technology" [ASBASJSM college of pharmacy Bella (Ropar) Punjab. India ;15-16 November 2014] **Received 1st Prize in poster presentation**
8. **Arun Sharma.** One day workshop on "Hands on Training Analytical Instrument Expertise-In collaboration with Industrial Experts" [Laureate Institute of Pharmacy, Kangra, H.P. India; 6 April 2014].